PROCEEDINGS OF

THE ROYAL SOCIETY.

Section B.—Biological Sciences.

*Further Results of the Experimental Treatment of Trypanosomiasis in Rats; being a Progress Report of a Committee of the Royal Society.**

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(Communicated by Sir Ray Lankester, K.C.B., F.R.S., Chairman of the Tropical Diseases Committee. Received October 28,—Read November 7, 1907.)

[Plate 1.]

The following results are a continuation of the work already described, and the experiments have been carried out upon rats inoculated with the same strains of Nagana and Surra:—

Condition of the Animals living at the Date of the Completion of the Tables in the former Paper.

Table I.—Nagana Rats treated with Atoxyl and Succinimide of Mercury.

No.	4	is still living and well	229	days	after inoculation.
„	7	„ „	222	„	„
„	10	„ „	178	„	„
„	15	„ „	164	„	„
„	21	„ „	63	„	„
„	6	died on the	214th	day	after inoculation.
„	12	„	110th	„	
„	16	„	81st	„	

* This Committee, which planned and supervised the investigations, was appointed by the Tropical Diseases Committee for the purposes of this experimental enquiry, and consists of the following members: Professor J. Rose Bradford, Colonel Bruce, Professor Cushny, and Mr. H. G. Plimmer. A preliminary summary of the results of the enquiry

In these rats the livers were found to be pale and fatty, and the kidneys degenerated: these were pale and fatty, with fibrous streaks, and the urine of Nos. 12 and 16, found in the bladder *post mortem*, contained albumen. They did not die of the disease, as no trace of trypanosomes could be found in either of them, but of the degenerative changes mentioned.

Table II.—Surra rats treated with Atoxyl and Succinimide of Mercury.

No.	5	died on the	206th	day after inoculation, of pneumonia.
„	9	„	169th	„ „ „
„	14	„	61st	„ from an unnoticed recurrence.
„	15	„	59th	„ from paralysis after three recurrences.
„	18	„	61st	„ .
„	19	„	59th	„ .
„	20	„	57th	„ after five recurrences, becoming finally axotyl-proof.

In Nos. 5 and 9 there was also evidence of fatty and fibrous degeneration of the kidneys.

Of these rats only in Nos. 14 and 20 was there any evidence that they died of the disease.

Table III.—Rats treated with Atoxyl and Mercury Sozoiodol.

No. 5 is still living and well, 208 days after inoculation.
„ 3 died on the 181st day after inoculation.

(The kidneys and liver of No. 3 were very fatty.)

Table V.—Rats treated with Atoxyl and Donovan's Solution.

No. 2 died on the 70th day after inoculation.
„ 6 „ 121st „ „

(The kidneys of No. 2 were degenerated, those of No. 6 markedly so, being pale in colour with yellow streaks, and very friable; there was albumen in the urine.)

Table VI.—Rats treated with Atoxyl and Iodipin.

No. 9 is still living and well 218 days after inoculation.
„ 10 died on the 141st day after inoculation.
„ 14 „ 178th „

(No. 14 had also 1·2 milligrammes of succinimide of mercury. The kidneys of this rat were degenerated, and it had albumen in its urine.)

From the above list it will be seen that the principal pathological lesion in those rats which have been treated with atoxyl and some compound of mercury and have lived for a very long time after inoculation, being, we think, cured of the disease, is a degeneration of the kidneys; and in most of

has already appeared in the 'Proceedings of the Royal Society' (B, vol. 79, 1907, pp. 505—516). By the courtesy of the governing body of the Lister Institute the investigations have been carried on in the laboratories of that institution.

these rats this was the only lesion found *post mortem*. This will be referred to later in mentioning further treatment with mercury.

Atoxyl and Calomel.

Twelve rats have been treated with atoxyl (three to five doses) and then with subcutaneous and intramuscular injections of calomel, in doses of 1 minim of Surgeon-Major Lambkin's formula. It is difficult in rats to make the injection into the muscles, and in all cases necrosis occurred at the site of the injection; no better result was attained than by treatment with atoxyl alone, with subsequent recurrences and death.

Atoxyl and Succinimide of Mercury.

Further experiments have been made with this combination, in which the dose of the mercury salt has been increased up to 1 milligramme. The 12 rats of this series are all dead, and showed acute kidney changes: inflammation, going on to necrosis of the epithelium, multiple hæmorrhages, etc.

Atoxyl and Donovan's Solution.

Nine further experiments have been made with this combination, also in larger doses; but these larger doses have been invariably fatal, with lesions both of the intestines and kidneys. The doses were arranged upon the basis of the doses recorded, with such good results, by Drs. Moore, Nierenstein, and Todd;* but one of us has received a letter, since the experiments were completed, from Dr. Nierenstein, stating that the Donovan's solution used in their experiments was diluted with an equal part of water.

Atoxyl and Liq. Hydrarg. Perchlor.

A series of 12 rats was treated with this combination on the lines laid down in the paper above referred to, by Drs. Moore, Nierenstein, and Todd. The results obtained by them gave much hope that this combination would be especially useful. But we have not been able to get such good results. Out of 12 rats so treated only one is alive at the 97th day, the others having died with acute renal lesions. Of course, really comparative results are always difficult to obtain, and in rats the individual equation, with regard to dosage, and to resistance both to drugs and to disease, is a very varying one. We have, for instance, found, with the rats we have used, that the white ones are more susceptible both to diseases other than trypanosomiasis and to drugs than the black and white ones are; and we find the grey are the least susceptible.

* "On the Treatment of Trypanosomiasis by Atoxyl . . . . . followed by a Mercurial Salt, etc.," 'Biochemical Journal,' vol. 2, Nos. 5 and 6.

From the above, considering both those experiments recorded in our former paper which have since ended fatally, and the more recent and—as regards dosage—bolder experiments, we are forced to the conclusion that, in small animals at any rate, mercury has not in our hands given altogether satisfactory results. Perhaps it may be a question of dosage; we have, however, tried to enlarge the range of dosage, as far as possible, from homœopathic doses to large ones, without attaining a large percentage of cures. If the dose of mercury be sufficient to aid the atoxyl, as in the cases brought forward from our last paper, we have found, in those cases which have died, chronic kidney, and in a less degree liver, lesions, which seem to be the late result of those more acute changes which we have found in those animals which have died earlier, either from disproportionate dosage or from some want of resistance to the drug.

Perhaps, in dealing with a more chronic trypanosome disease such as Sleeping Sickness in man, the results would be more favourable. We have, for instance, two Sleeping Sickness rats, inoculated on April 15, which have been treated with quite small doses of atoxyl and succinimide of mercury, and in which no trypanosomes have been found since May 4; they appear to be quite well. We have also another Sleeping Sickness rat, inoculated on May 6, which has been treated only with atoxyl, and in which no trypanosomes have been found since May 28. But in the more acute forms of trypanosomiasis, such as Nagana and Surra, the method of treatment by atoxyl and some form of mercury has, in our experience, almost invariably led to degenerative lesions, principally in the kidneys, which has been a cause of death long after any trace of trypanosomes could be found, when we have believed that the animal has been quite cured of the initial disease.

Tiodine.

A few rats were treated with this substance, which is thiosinaminethyl-iodide ($C_6SN_2H_{13}I$). In doses of 10 minims it is immediately fatal to rats, although it is stated to be non-poisonous to man, and in doses of 5 minims caused death within 24 hours; in smaller doses, alone or in combination with atoxyl, it had no influence on the disease.

Certain Antimony Compounds.

The treatment with arsenic compounds was, as has been stated, attended with only partial success. Professor Cushny, F.R.S., who has advised us on pharmacological matters throughout these investigations, sent us for trial a weak combination of glycine and antimony, attempts to form an antimony compound analogous to atoxyl having failed. When injected into

inoculated rats, the antimony glycine was found to possess, in a less degree, the power of the antimony compounds described below; it reduced the number of trypanosomes, and caused their disappearance from the blood for a time, if they were not very numerous.

But the combination was difficult to make, varied in strength, and its solution was very dilute, and its use was abandoned when it was found that the other antimony compounds, described below, were so much more effectual.

Potassium Antimonyl Tartrate.

Potassium antimonyl tartrate (tartar emetic), which is easily soluble, was then tried. In doses of 1 c.c. of a 1 per cent. solution it proved fatal within 24 hours to four inoculated rats of weights varying from 190 to 225 grammes, but it was noticed that the trypanosomes had greatly diminished in number. It was also noticed that the rats appeared to be ill and faint for a short time after the injection: they were unsteady and sometimes rolled about. This was at the time attributed to the depressing effect of the potassium in the compound, and suggested the making and using of the substance described below, with which all of our experiments have so far been done. The question of the effect of this particular salt on the rats themselves will be mentioned later. Experiments are in progress for the purpose of comparing the actions of the potassium and sodium compounds: the latter would seem to possess some theoretical advantage, especially in treating larger animals, when the dose will have to be proportionally larger.

Sodium Antimonyl Tartrate.

Through the kindness of Dr. R. H. Aders Plimmer, of University College, we have been able to obtain and use this compound, which is the sodium salt corresponding to potassium antimonyl tartrate. He has prepared a quantity of the pure substance for us, in crystalline form, and has written the appended Note* upon its chemistry.

This substance in 1 per cent. solution is that which, of all the various bodies mentioned in these papers, including atoxyl, has the most marked and remarkable influence upon trypanosomes in the living body. Although our experiments with it are not many, nor of long duration, the results so far seemed sufficient to induce us to call the attention of other workers in this field to it.

The injection of this compound causes no pain, nor does it produce any inflammation of the tissues; and the results of the injection are very striking. The trypanosomes disappear with great rapidity from the blood, and in the

* See Note at end of paper, p. 11.

majority of the cases treated so far there has been no recurrence; and inoculations made from animals which have been killed, or have died, have been invariably negative. It acts much more quickly than atoxyl, and its dose is very much smaller, and it does not produce any undesirable effects on the animals, and recurrences are very much less frequent than is the case when atoxyl has been used.

We have used a 1 per cent. solution of the solid salt, but this is quickly invaded by moulds, and needs the addition of a crystal of thymol, or of 0·25 per cent. of formalin. The question of dosage is still under observation. We have tried many ways, and at present we are inclined to think that a full dose (*e.g.*, 0·5 c.c. of a 1 per cent. solution for a rat of 200 grammes or over) should be given when the trypanosomes are fairly plentiful in the blood, and then repeated at intervals of one, two, and three days, up to about four doses, and thereafter in weekly doses for a month. But we have good results in cases in which a dose has been given on four successive days, also when given every other day, and so on up to once every five days, without any recurrence up to as many as 52 days; but of two cases dosed at five-day intervals, one has recurred and one has not. As regards the quantity which can be taken, we have one rat of 130 grammes weight which has taken 0·5 c.c. of a 1 per cent. solution on the 3rd day (when trypanosomes were plentiful in the blood), and again on the 5th, 0·29 c.c. on the 6th and 7th days, and 0·25 c.c. on the 8th, 9th, 10th, 12th, 13th, 14th, 15th, 16th, 17th, and 19th days, and it is still living and well on the 43rd day, and has had no recurrence. When it is given in a full dose for the first time to rats whose blood is swarming with trypanosomes, the rat generally becomes very restless, and rolls about; its respirations become very quick, and it appears to be ill. These symptoms were noticed in the initial experiments with tartar emetic, and were attributed to the potassium contained in it, but we think that they are more probably due to the changes in the blood caused by the destruction or solution of the trypanosomes, which occurs very rapidly, as they do not occur after the second dose, when there are no trypanosomes. Recovery has, so far, always taken place from this condition, but it would seem advisable, in cases where the first dose is delayed until the blood is swarming with trypanosomes, to give the dose in two halves at intervals of a few hours.

The quickness of the action of sodium antimonyl tartrate is very remarkable. In one rat, whose blood was swarming with trypanosomes, a dose of 0·35 c.c. of a 1 per cent. solution caused their entire disappearance from the blood within half an hour; and in two other cases, in which the blood contained very large numbers of trypanosomes, after injection of 0·33 c.c. only a few could be found at the end of half an hour, and in one after an hour none could

be found, and in the other only one in an ordinary blood preparation (see Plate 1). In these cases a few trypanosomes can sometimes be found in the liver, and these are extremely active and in no way inconvenienced by the drug; whether these are the forms which can persist, and need to be tired out by successive doses, we cannot say at present, but their extreme activity, when all the others have disappeared, is suggestive. We have up to the present not detected any morphological differences in them.

A striking instance of the power of this compound over trypanosomes is seen in the case of a guinea-pig which was inoculated with *Trypanosoma Gambiense* on April 9. From July 3 to September 16, trypanosomes were present in the blood, latterly in quantity, and the animal was dying on September 16; its eyelids were œdematous and nearly closed; it had œdema of the genitals and anus, and a discharge of bloody mucus from the rectum, and its hair was coming out in large patches. At this date—September 16—it was given 0·5 c.c. of a 1 per cent. solution; on the 17th the trypanosomes had entirely disappeared, and 0·75 c.c. was given; on the 19th the animal to all appearances was quite well, and on this day, and on 21st and 26th, 1 c.c. was given. The œdema disappeared and it continued to look well, and showed no more trypanosomes. It lived until October 14, when it died; *post mortem* the organs were congested and the kidneys were inflamed and the urine in the bladder contained albumen. The fact that the guinea-pig was moribund when the treatment was commenced may reasonably account for the pathological condition.

The following table shows the general results, as obtained so far, of the treatment with sodium antimonyl tartrate. Of the 39 rats enumerated in this table, the first 3 had been treated at first with antimony glycine; and of the 11 of the remaining 36 which have died, 6 did not die of the disease, and there remain alive and well 3 of 52 days, 1 of 49, 7 of 44, 8 of 43, 4 of 31, and 2 of 21; and of these 25, 23 have had no recurrence. It will be seen that 8 of the rats (4 Nagana and 4 Surra) have been treated with mercury in addition to the sodium antimonyl tartrate, in order to see if these obtained thereby any advantage over those not so treated. So far as we can tell at present, there seems to be no obvious advantage: one of the Nagana rats treated with liq. hydrarg. perchlor. has had three recurrences, and one Surra rat treated with succinimide of mercury has died after one recurrence.

Sodium Arsenyl Tartrate.

As the results with the sodium antimonyl tartrate were so definite, it seemed worth while to investigate the effect of the corresponding arsenic compound.

Table of Nagana and Surra Rats treated with Sodium Antimonyl Tartrate. Average duration of untreated diseases 5·5 and 6·9 days respectively.

Disease.	No.	Weight in grammes.	Total amount of 1 per cent. sodium antimonyl tartrate in c.c.	Result to Oct. 24.	Recurrences.	Remarks.
Nagana ...	1	150	6·2	Died on 49th day...	2	Antimony glycine had been given at first, sodium antimonyl tartrate on first recurrence.
,,	2	200	0·83	,, 25th ,, ...	1	,, ,, ,, No trypanosomes were found for 12 days before or at death.
,,	3	170	1·75	,, 35th ,, ...	1	Antimony glycine had been given at first, sodium antimonyl tartrate on first recurrence. No trypanosomes found for 10 days before, or at death, and a mouse inoculated with bone marrow did not take the disease.
,,	4	115	2·76	,, 38th ,, ...	1	No trypanosomes found for 16 days before, or at death. Rat became paralysed.
,,	5	175	1·35	,, 16th ,, ...	1	Probably too small a dose.
,,	6	125	1·0	,, 18th ,, ...	2	,, ,,
,,	7	200	3·0	*Living 52 days*.....	0	
,,	8	150	2·5	,, *52* ,, ...	0	
,,	9	250	2·6	,, *52* ,, ...	0	
,,	10	215	3·5	,, *49* ,, ...	0	
,,	11	125	2·6	Died on 22nd day...	1	Died of septicæmia from sloughing tail, not of disease. No trypanosomes found.
,,	12	195	3·25	,, 19th ,, ...	0	,, ,, ,,
,,	13	175	1·85	,, 40th ,, ...	0	Died of septicæmia from an abdominal abscess, not of disease. No trypanosomes found.
,,	14	110	1·9	*Living 43 days*.....	0	
,,	15	130	3·6	,, *43* ,, ...	0	This rat had 14 doses in 19 days, *vide* p. 6.
,,	16	155	1·5	,, *43* ,, ...	0	
,,	17	120	1·15	,, *43* ,, ...	0	
,,	18	225	3·75	Died on 31st day ...	2	No trypanosomes found *post mortem*.
,,	19	140	1·48	*Living 43 days*.....	0	

,,	20	125	3·75	,, *43* ,, ...	3	Had 2 × 0·5 c.c. liq. hydrarg. perchlor. after first four doses of sodium antimonyl tartrate.
,,	21	150	1·5	,, *43* ,, ...	0	
,,	22	125	1·5	Died on 27th day...	1	Had 2 × 0·25 mg. succinimide of mercury after first four doses of sodium antimonyl tartrate.
,,	23	125	1·1	*Living 43 days*.....	0	Did not die of disease. No trypanosomes found.
,,	24	150	2·5	Died on 28th day...	1	
,,	25	115	1·8	*Living 31 days*.....	0	
,,	26	125	2·1	,, *31* ,, ...	0	
,,	27	125	2·1	,, *31* ,, ...	0	
,,	28	140	2·0	,, *31* ,, ...	0	
,,	29	190	1·5	,, *21* ,, ...	0	
,,	30	150	1·4	,, *21* ,, ...	0	
Surra	31	275	5·0	*Living 44 days*.....	1	
,,	32	175	1·9	,, *44* ,, ...	0	
,,	33	175	1·5	,, *44* ,, ...	0	Had 2 × 0·5 c.c. liq. hydrarg. perchlor. after four doses of sodium antimonyl tartrate.
,,	34	175	1·5	,, *44* ,, ...	0	
,,	35	200	2·0	,, *44* ,, ...	0	Had 2 × 0·25 mg. succinimide of mercury after four doses of sodium antimonyl tartrate.
,,	36	150	1·5	,, *44* ,, ...	0	
,,	37	175	3·4	Died on 37th day...	0	Rat had eaten part of its fore-leg and tail, and was killed. No trypanosomes had been seen since the fourth day of the disease (and first of treatment). None were found *post mortem*, and two rats were inoculated with gland and marrow, with negative result.
,,	38	250	4·0	*Living 44 days*.....	0	Did not die of disease. No trypanosomes found after fourth day of disease (and first of treatment).
,,	39	250	2·0	Died on 11th day...	0	

Sodium Arsenyl Tartrate or Sodium Tartrarsenite.—This compound was investigated by Henderson and Ewing* in 1895, who gave it the formula $AsONaC_4H_4O_6.2\frac{1}{2}H_2O$.

It was prepared for these experiments by dissolving one equivalent of arsenious oxide in two equivalents of acid sodium tartrate, filtering, evaporating to a small volume, and adding alcohol till crystallisation commenced; on cooling, the substance crystallised out.

This substance does not seem to be anything like so effective as the antimony compound. Of five rats which have been treated with it, four died between the 12th and 24th days, and three of these had a recurrence; one is still living on the 21st day, but we think that this is probably due to the sodium antimonyl tartrate which was given after a recurrence, in the same way as if from the beginning of the disease.

A mixture of equal parts of a 1 per cent. solution of sodium antimonyl tartrate and of sodium arsenyl tartrate has been tried on six rats. One died on the 14th day and the five others are still living on the 21st day without any recurrence.

Immunity.

With a view of ascertaining what amount of immunity, if any, had been conferred on an animal which we considered to be cured, a Nagana rat was taken which was inoculated on May 13, and had been afterwards successfully treated with atoxyl and succinimide of mercury, and in which no trypanosomes had been found since it had its first dose on May 16, when the trypanosomes were very plentiful in the blood. On October 7, the 147th day, the rat was re-inoculated from another Nagana rat, and on the 11th trypanosomes were present in numbers in the blood; a dose of sodium antimonyl tartrate was given and no trypanosomes have been seen since the 12th. This seems to point to the fact that no immunity is conferred.

DESCRIPTION OF PLATE.

The microphotographs were made from rough blood-preparations, with a low-power objective (Zeiss 8 mm.), in order to demonstrate the rapid disappearance of the trypanosomes from the blood after administration of sodium antimonyl tartrate.

Fig. 1 shows the blood of a Nagana rat, 4 days after inoculation, *before treatment.*

Fig. 2 shows the blood from the same rat *half an hour* after the injection of 0·35 c.c. of a 1 per cent. solution of sodium antimonyl tartrate.

Fig. 3 shows the blood from the same rat *one hour* after administration of the above dose

* Henderson and Ewing, 'Chem. Soc. Trans.,' 1895, vol. 67, p. 103.

FIG. 1.

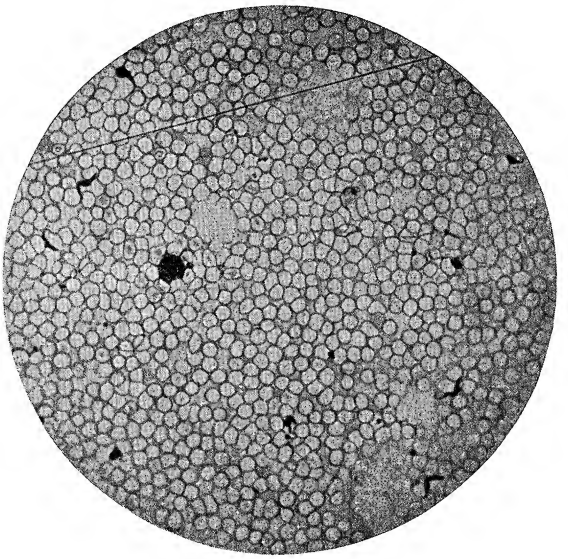

FIG. 2.

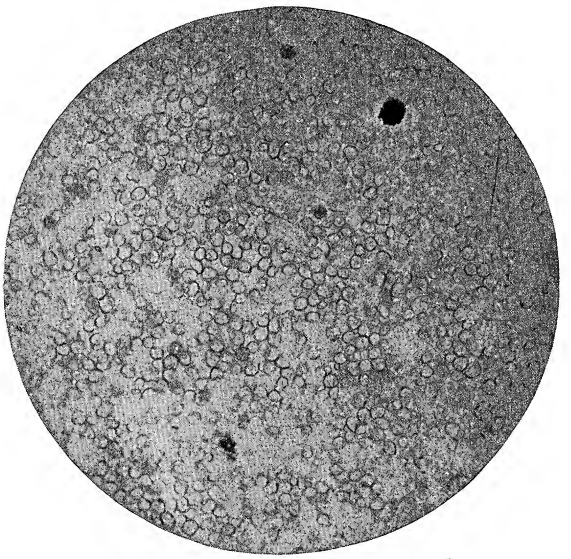

FIG. 3.

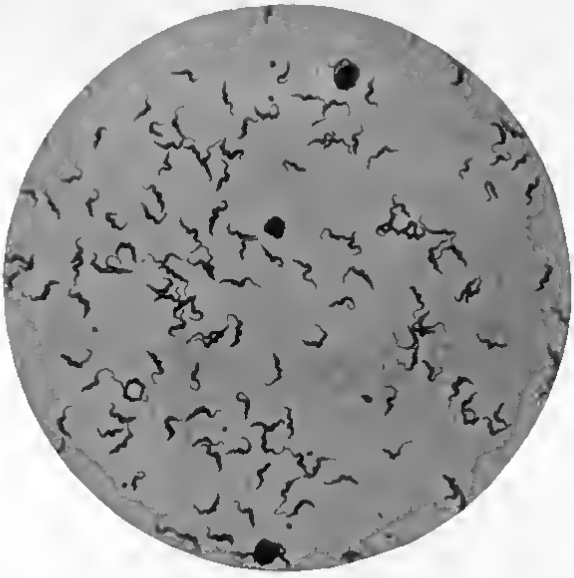

FIG. 1.

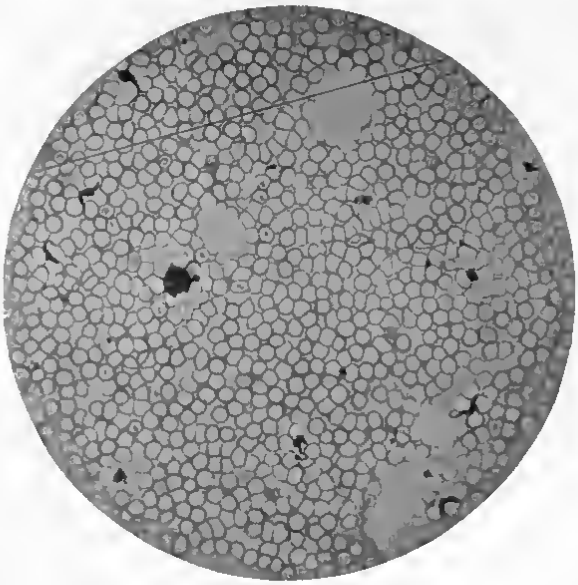

FIG. 2.

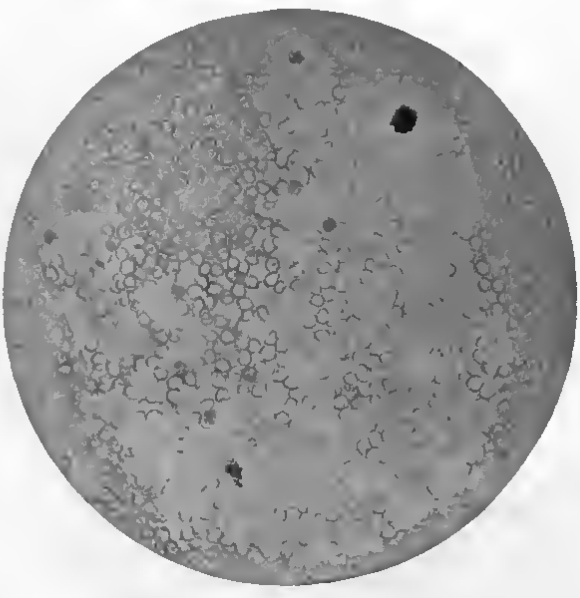

FIG. 3.